EVALUATION OF THE ANTI-OXIDANT ETHYLENE DIUREA (EDU) AS A PROTECTANT AGAINST OZONE EFFECTS ON CROPS

(Growth Chamber Trials)

Alberta Environment

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EVALUATION OF THE ANTI-OXIDANT ETHYLENE DIUREA (EDU) AS A PROTECTANT AGAINST OZONE EFFECTS ON CROPS

(Growth Chamber Trials)

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FOREWORD

The Air Research User's Group of Alberta Environment previously managed a field research project that studied the ability of foliarly applied ethylene diurea (EDU) to protect crops against detrimental effects of ozone. Ethylene diurea is an antioxidant that was developed in the 1970's to protect plant tissue from ozone injury; however, the nature of the protective effect is unclear. In the previous study several growth parameters, including plant height and seed yield were significantly increased when EDU was applied to the foliage. The magnitude of the observed increases varied by species and may have resulted from species specific effects. In order to determine if the observed increases in growth were a result of protection against ozone injury, or due to physiological effects of EDU unrelated to ozone, further research was required. The project described in this report made use of controlled-environment exposure studies, to determine if the effects observed in the field trial were a result of EDU protection against ozone or plant growth promoting effects of EDU.

Laura Blair
Project Coordinator
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SUMMARY

Results of a field study conducted in the summer of 2000 showed that when the antioxidant EDU was applied to crops, there were increases in several parameters. The next step was to verify whether the effects of EDU resulted from EDU-mediated protection against O_3 or from plant growth promoting effects of EDU that are independent of O_3. A series of controlled-environment trials were designed to test this hypothesis. The objectives of this project were to:

- modify an existing gas exposure system to expose plants to O_3 and test the stability and reliability of the system,
- perform O_3 exposure trials using wheat, barley, field pea and canola to observe the effects of O_3, and
- test whether effects of EDU can be attributed to protection against O_3 injury or to plant growth promoting effects that are independent of O_3.

The gas exposure system described by Li and Archambault (1999) was modified for use as an O_3 exposure system. This required the installation of an O_3 delivery system and a monitoring system. When the exposure system was judged to be functioning reliably, preliminary trials were conducted using radish plants to further test the exposure system's stability.

Stippling and flecking of radish leaves began after two weeks of exposure to O_3 followed by the appearance of mottled bleached areas that became red as the symptoms progressed. In wheat and barley, symptoms of O_3 injury appeared as mottled chlorotic areas that led to complete chlorosis, necrosis and eventually to abscission. The awns of plants exposed to O_3 developed a deep brownish orange colour. In field pea, tendril curl developed after approximately two weeks of exposure. No visual symptoms of O_3 injury were observed in canola plants exposed to O_3.

Treatment of plants of cv. Cherry Belle with EDU reduced the number of plants exhibiting O_3 injury while treatment with EDU provided complete protection to plants of cv. Early Scarlet Globe from visual symptoms of O_3 injury. Leaf area of both cultivars was lower in plants grown at elevated O_3 while leaf biomass was only depressed in cv. Early Scarlet Globe.

In wheat and barley, no effects of O_3 and EDU on plant height were observed. In wheat cv. CDC Teal, above-ground biomass was decreased by exposure to O_3. Effects of EDU on seed weight and seed number were significant. Ozone caused a decrease in head weights, seed number and seed yield in all treatments. In wheat cv. AC Taber, above-ground biomass was decreased by exposure to O_3. Number of seed heads increased in plants exposed to O_3 but weight of heads, number of seeds, seed yield and weight per thousand seeds decreased. Alleviation of O_3 effects on head and seed weights were significant. In barley cv. AC Lacombe, no effects of O_3 and EDU on above-ground biomass were observed. Weights of seed heads, number of seeds and seed yields were greater in plants grown at elevated O_3 without effects of EDU. Treatment with EDU caused increases in number of seed heads, weights of seed heads, number of seeds and seed yields at low O_3 levels. Ozone had no effect on plant height and above-ground biomass in field pea while seed yield was decreased. EDU application, with or without O_3, had no statistically

significant effects on field pea for any of the parameters measured. While in canola, plant height and above-ground biomass were not affected by O_3, seed number and yield increased in cv. Quantum and decreased in cv. Sprint. Effects of EDU were observed on biomass of cv. Quantum and weight per thousand seed of cv. Sprint.

Of the field crops studied, wheat cv. CDC Teal was found to be most sensitive to EDU while AC Taber was most sensitive to ozone. No compelling evidence of growth promoting effects of EDU in the absence of O_3 was found. It is apparent from this study that the effects of EDU can be species- and cultivar-specific. Archambault *et al.* (2000) have also suggested that the dose, frequency and mode of application of EDU are critical and that one must take into consideration the length and frequency of ozone exposure as well as environmental conditions in developing protocols for the use of EDU for the quantification of the effects of ozone on vegetation.

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1.0 INTRODUCTION

Ozone is an important phytotoxic gaseous pollutant in Canada, the USA and many other industrialized countries. Attempts to protect plants using various chemical substances have been made. Protection may be achieved by coating the leaf surface and providing physical and/or chemical protection, through the alteration of gas exchange or through the alteration of plant metabolism. Several studies suggest that the application of certain chemical protectants against O_3 might be a reliable means by which to assess crop effects under field conditions. Manning and Krupa (1992) presented a list of examples of chemicals used to protect plants from O_3 injury with references spanning from 1960 to 1991. While many chemicals have been shown to convey partial or total protection against O_3 injury, many are ineffective and have unacceptable side-effects rendering them of little value for the purpose of crop effects assessments in the field (Archambault *et al.* 2000).

Through the late 1980's and into the 1990's EDU (ethylene diurea) has been used under a number of conditions as a protectant from O_3 injury (Archambault *et al.* 2000). Researchers reported that EDU reduced and/or delayed the appearance of O_3 damage to developing foliage and delayed plant senescence and leaf abscission. These findings showed promise for the use of EDU as a general protectant against O_3 damage but in order to be useful as a tool for the determination of crop losses due to O_3 exposure, it was also necessary to verify whether EDU caused side-effects in the absence of O_3. The possible side-effects of EDU were discussed by Legassicke and Ormrod (1981) and Foster *et al.* (1983).

Studies have been conducted to establish optimized protocols for the use of EDU in programs aiming at the quantification of the effects of O_3 on vegetation and to understand the process by which EDU conveys protection against O_3. The effects of EDU are species- and sometimes cultivar-specific, and the concentration, frequency and mode of application are critical considerations. In designing an EDU treatment regime, it is also important to consider the length and frequency of O_3 exposure as well as environmental conditions (Manning 1995).

Based on the literature, in the summer of 2000 a protocol making use of EDU as an antioxidant was designed and implemented at a site near the city limits of Fort Saskatchewan, Alberta, an area having elevated O_3 levels relative to other areas of the province. Results showed that when EDU was applied there were increases in several parameters including plant height and seed yield. The next step was to verify whether the effects of EDU observed in the field resulted from EDU-mediated protection against O_3 or from plant growth promoting effects of EDU that are independent of O_3. Therefore, a series of controlled-environment trials were designed to test this hypothesis. The objectives of this project were to:

- modify an existing gas exposure system to expose plants to O_3 and test the stability and reliability of the system,
- perform O_3 exposure trials using wheat, barley, field pea and canola to observe the effects of O_3, and
- test whether effects of EDU can be attributed to protection against O_3 injury or to plant growth promoting effects that are independent of O_3.

The results of this study provide an indication of the usefulness of EDU in a large-scale examination of O_3 effects on agricultural crops in Alberta.

2.0 METHODS AND MATERIALS

2.1 PLANT GROWTH CONDITIONS

2.1.1 Growth Chamber Conditions

A growth chamber gas exposure system previously constructed to expose plants to ethylene (Li and Archambault, 1999) was modified to add O_3 exposure and monitoring capabilities. Ozone generators were used to inject O_3 into a stream of charcoal-filtered air that fed growth chambers. Ozone was produced from ultra high purity oxygen (BOC) from a cylinder. Rotameters were used to control the flow of oxygen to each O_3 generator, thereby allowing for control of O_3 concentrations within the growth chambers. Ozone levels were monitored approximately twice daily by selecting a sampling stream using a manually-operated manifold drawing air through Teflon sampling lines to a UV O_3 analyzer and manually recording O_3 levels. Mean O_3 levels were calculated for the exposure period. Light intensities at mid-canopy were maintained at approximately 300 µmols m^{-2} s^{-1}. A photoperiod of 16 hours light and 8 hours dark was maintained using a combination of fluorescent lights and incandescent lights. Temperatures were maintained at 23°C daytime and 15°C nighttime and were monitored using chart recorders. Relative humidity was maintained at approximately 50%

2.1.2 Growth Containers and Growth Medium

In the first radish trial, plants were grown in wells in trays of 25 containing approximately 125 mL of soilless mix. In the second trial, plants were grown in 6" pots containing approximately 1.25 Kg of soilless mix at a density of 3 plants per pot. In all crop experiments plants were grown in 6" pots containing approximately 1.25 Kg of soilless mix at densities of three plants per pot for cereals and field pea and 8 plants per pot for canola. Crop plants were fertilized as required using ammonium nitrate, mono-potassium sulphate and a modified Hoagland's solution containing only micronutrients.

2.2 CROP SELECTION, EDU TREATMENTS AND EXPERIMENTAL DESIGN

2.2.1 Crop Selection

Two cultivars of radish (*Raphinus sativus* L.) differing in sensitivity to O_3 were selected for preliminary studies used to test the exposure system and to compare results obtained in the present study to those from the literature. The cultivars were: Cherry Belle (sensitive) and Early Scarlet Globe (resistant). Sensitivity of these cultivars to O_3 was based on the appearance of visual symptoms such as leaf lesions.

Cultivars of four regionally common crops were selected for this study. They were:

- wheat (*Triticum aestivum*) cvs. CDC Teal and AC Taber,
- barley (*Hordeum vulgare*) cvs. AC Lacombe,
- canola (*Brassica napus)* cvs. Sprint and Quantum, and
- field pea (*Pisum sativum*) cv. Carrera.

2.2.2 Treatments

In the first radish trial, EDU was applied to run-off weekly at concentrations of either 0, 75 or 150 ppm using a paint sprayer. In the second radish trial, EDU was applied as a single soil drench of 100 mL per pot containing either 0.2025 g or 0.6089 g of EDU. In the crop experiments, EDU was applied to run-off using a spray chamber at intervals of approximately 10 days from the time the plants were 8 – 10 cm in height to maturity giving 4-5 applications. Concentrations were selected based on effects observed in the field trial (Archambault and Li, 2001) and in cases where the lowest concentration used in the field (150 ppm) had greatest effects, a treatment of 75 ppm was used to determine whether greater protection could be achieved. EDU concentrations were: 0, 75 and 150 ppm for wheat, barley and canola cv. Quantum; 0, 150 and 300 ppm for canola cv. Sprint and field pea.

2.2.3 Experimental Design

In the first radish trial, the experimental design was:

2 cultivars x 2 O_3 levels x 3 EDU treatments x 3 replicates (trays of 25)

In the second radish trial, the experimental design was:

2 cultivars x 2 O_3 levels x 2 EDU treatments x 4 replicates (pots)

In the crop trials, the experimental design was:

1 or cultivars x 2 O_3 levels x 3 EDU treatments x 4 replicates (pots)

2.3 ASSESSMENTS

2.3.1 Radish experiments

Visual symptoms of O_3 injury were photographed as they developed during exposure. Plants were assessed and harvested when they reached the early flowering stage. In the first trial, the number of plants per treatment with leaf lesions was recorded. Leaves of the six largest plants (6 out of 25) per treatment were harvested, leaf area per plant measured using a leaf area meter (LI-COR), and the leaves were dried in a drying oven at 70°C and weighed. In the second trial, the number of plants per treatment with leaf lesions was recorded. Leaves were harvested, leaf area per pot (3 plants) measured using a leaf area meter (LI-COR), and the leaves were dried in a drying oven at 70°C and weighed. Hypocotyls and roots were washed, dried and weighed together.

2.3.2 Crop Experiments

Plants were examined weekly throughout the experiment for symptoms of O_3 damage and EDU toxicity and/or protection. Photographic records were kept. The heights of the tallest plants in each pot were recorded prior to harvest. Above-ground biomass was harvested, dried in an oven at 70°C and weighed. Samples were threshed and the seeds were extracted, weighed and counted, and the weight per thousand seeds was calculated.

2.4 STATISTICAL ANALYSIS

Data were entered into Excel worksheets and means, standard deviations and standard errors were computed. All data were analyzed using SAS version 2.0. Parameters were tested for main effects of EDU, ozone and for EDU x ozone interactions. In addition, effects of EDU within each ozone treatment were tested to determine if EDU alleviated ozone effects.

3.0 RESULTS AND DISCUSSION

3.1 EXPOSURE SYSTEM AND O_3 LEVELS

The gas exposure system described by Li and Archambault (1999) was modified for use as an O_3 exposure system. This required the installation of an O_3 delivery system and an O_3 monitoring system. Ozone levels were set by adjusting the flow of ultra high purity oxygen through O_3 generators using rotameters and monitoring the O_3 levels in the chambers using an O_3 analyzer calibrated quarterly by technicians at Alberta Environment. When the exposure system was judged to be functioning adequately, preliminary trials were conducted using radish plants to further test the exposure systems stability. Radishes were used because of there short life cycle, thereby expediting tests. This would permit comparison of the results to a vast literature and provide additional information on the performance of the system prior to undertaking long exposures, upwards of three months, using crops. In the first trial using radish, target O_3 levels were 0 ppb controls and 50 ppb treatments. Average ($\pm$SE, n=43) O_3 levels were 0 (0), 0 (0), 0 (0), 38.7 (1.0), 54.8 (1.4) and 50.5 (1.4) over the 28-day exposure period in the six exposure chambers. Ozone levels required adjustment for future experiments. Leaks in ozonators were the cause of off-target concentrations. The problem was rectified by re-sealing the ozonators. Ozone levels were closer to target levels in subsequent experiments. For cereals, target O_3 levels were 0 ppb controls and 50 ppb treatments. Average ($\pm$SE) O_3 levels were 0.5 (0.6) and 50.5 (11.1) for barley cv. AC Lacombe, 0.6 (0.7) and 50.0 (10.3) for wheat cv. CDC Teal and 0.5 (0.5) and 52.0 (7.9) for wheat cv. AC Taber. Having observed few effects of EDU in cereals exposed to O_3 at 50 ppb we believed that the effects of ozone were too severe for EDU to be effective in protecting plants from injury; therefore, O_3 levels were decreased in subsequent experiments. For field pea and canola, target O_3 levels were 0 ppb controls and 35 ppb treatments. Average O_3 levels were 0.5 (0.9) and 35.5 (8.0) for field pea cv. Carrera, 0.3 (0.7) and 31.9 (9.0) for canola cv. Sprint and 0.2 (0.4) and 34.9 (10.2) for canola cv. Quantum.

3.2 VISUAL SYMPTOMS OF O_3 INJURY

Radish leaves began to show symptoms of O_3 injury after approximately two weeks of exposure at approximately 50 ppb. Stippling and flecking of leaves was followed by the appearance of mottled bleached areas that became red as the symptoms progressed (Figure 1). These symptoms are consistent with those reported in the literature (Krupa *et al.* 2000). Leaf margin curl and necrosis were also observed. The symptoms were alleviated by treatment with EDU and in some occasions EDU prevented the development of visual symptoms altogether (Figure 1, Table 1). Based on visual symptoms and in agreement with Kostka-Rick *et al.* (1993), cv. Early Scarlet Globe is more resistant to O_3 injury than cv. Cherry Belle. In wheat and barley, symptoms of O_3 injury appeared as mottled chlorotic areas on both young and old leaves (Figure 2) that led to complete chlorosis, necrosis and eventually to abscission. At maturity, the awns of plants exposed to O_3 had developed a deep brownish orange colour and plants were nearly completely necrotic; whereas, control plants had green leaves remaining. In field pea, tendril curl developed after approximately two weeks of exposure at 35 ppb. No visual symptoms of O_3 injury were observed in canola plants exposed to 35 ppb O_3 throughout their life cycle.

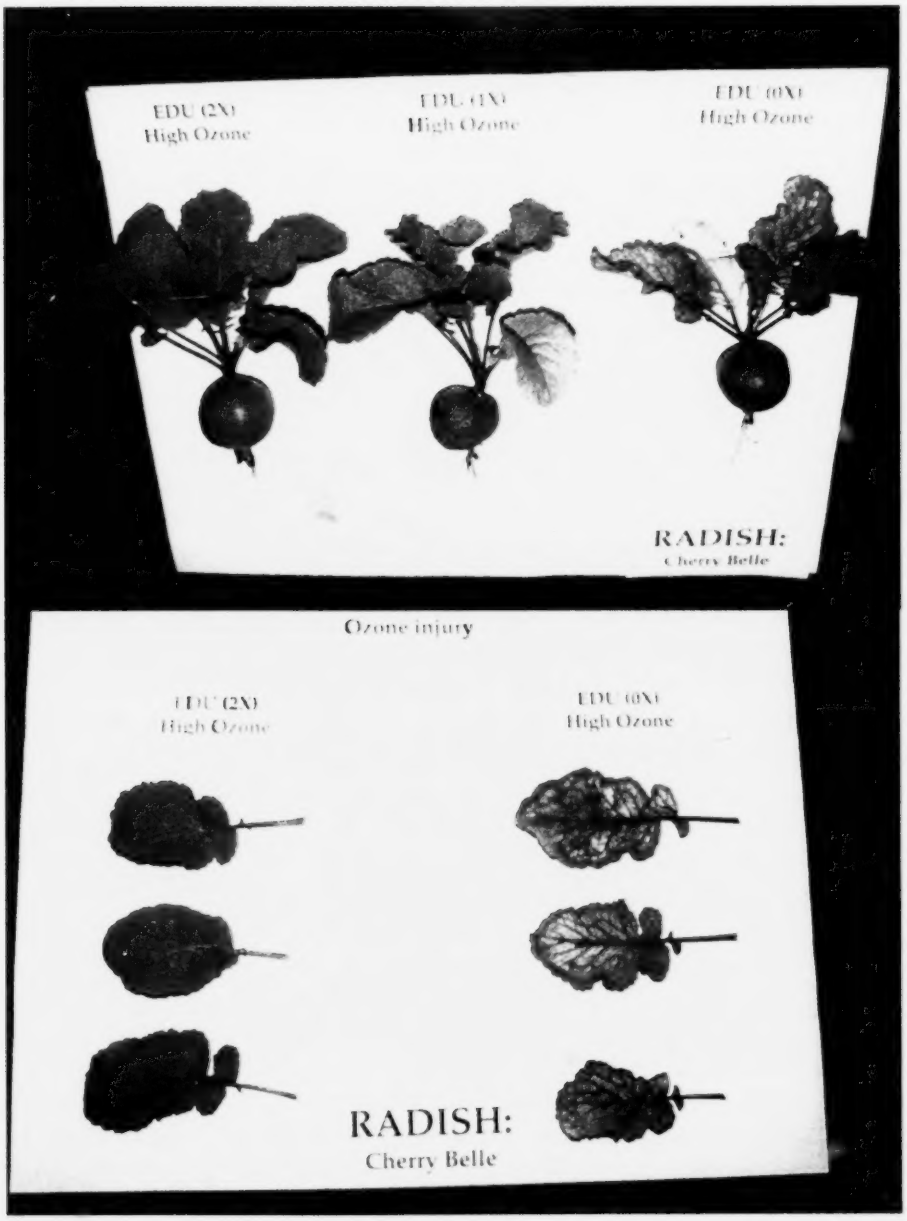

Figure 1 **Symptoms of O_3 injury in radish cv. Cherry Belle exposed to approximately 50 ppb O_3 for 18 days with or without EDU treatment**

Table 1 **Percentage of plants per treatment exhibiting visual symptoms of O_3 injury, total leaf area of six largest plants, total leaf biomass of six largest plants for radish cv. Cherry Belle grown in charcoal filtered air (0 ppb) or at approximately 50 ppb O_3 and treated with EDU at either 0, 75 or 150 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	% of plants with O_3 injury	Leaf area (cm^2)	Leaf Biomass (g)
0	0	0 ± 0	407.3 ± 29.0	2.38 ± 0.09
	75	0 ± 0	414.3 ± 14.5	2.33 ± 0.19
	150	0 ± 0	430.8 ± 17.5	2.71 ± 0.23
50	0	20 ± 8.3	382.2 ± 16.1	2.32 ± 0.13
	75	8 ± 4.0	359.8 ± 14.0	2.64 ± 0.16
	150	4 ± 2.3	393.5 ± 22.0	2.44 ± 0.26

Values are means ± SE. N = 3.

3.3 EFFECTS OF O_3 AND EDU

3.3.1 Radish Trials

Trials using radish, a widely studied species, were conducted in order to test the performance of the exposure system and to ensure that results found in the literature could be repeated as evidence of proper function of the exposure system. Results show varietal differences in sensitivity to O_3 measured as number of plants with O_3 lesions (Tables 1 and 2) in agreement with results found in the literature (Kostka-Rick *et al.* 1993). A greater number of radish plants exhibited visual symptoms of O_3 injury when plants were grown in pots in groups of three than when they were grown in soil plugs in trays (Tables 1, 2, 3 and 4). This may have been caused by the greater density of plants grown in trays, thereby affecting the interception of O_3 from air. Twenty percent of plants of cv. Cherry Belle and 5% of plants of cv. Early Scarlet Globe grown in trays exhibited symptoms of O_3 injury (Tables 1 and 2). Treatment of plants of cv. Cherry Belle with 150 ppm EDU reduced the number of plants exhibiting O_3 injury from 20% to 4% while treatment with 75 ppm EDU was sufficient to provide complete protection to plants of cv. Early Scarlet Globe from visual symptoms of O_3 injury and alleviation of O_3 symptoms by EDU treatment was significant at $P \geq 0.90$. Leaf area of both cultivars was lower in plants grown at elevated O_3 while leaf biomass was only depressed in cv. Early Scarlet Globe (Tables 1 and 2). Results show that sensitivity rankings are parameter-specific.

In radish plants grown in pots, all plants exhibited symptoms of O_3 injury at elevated O_3 in the absence of EDU treatment (Tables 3 and 4). In both cultivars EDU suppressed visual symptoms of O_3 injury and symptoms were completely eliminated at the highest rate of application of EDU. In both cultivars EDU effects were statistically significant at $P \geq 0.95$.

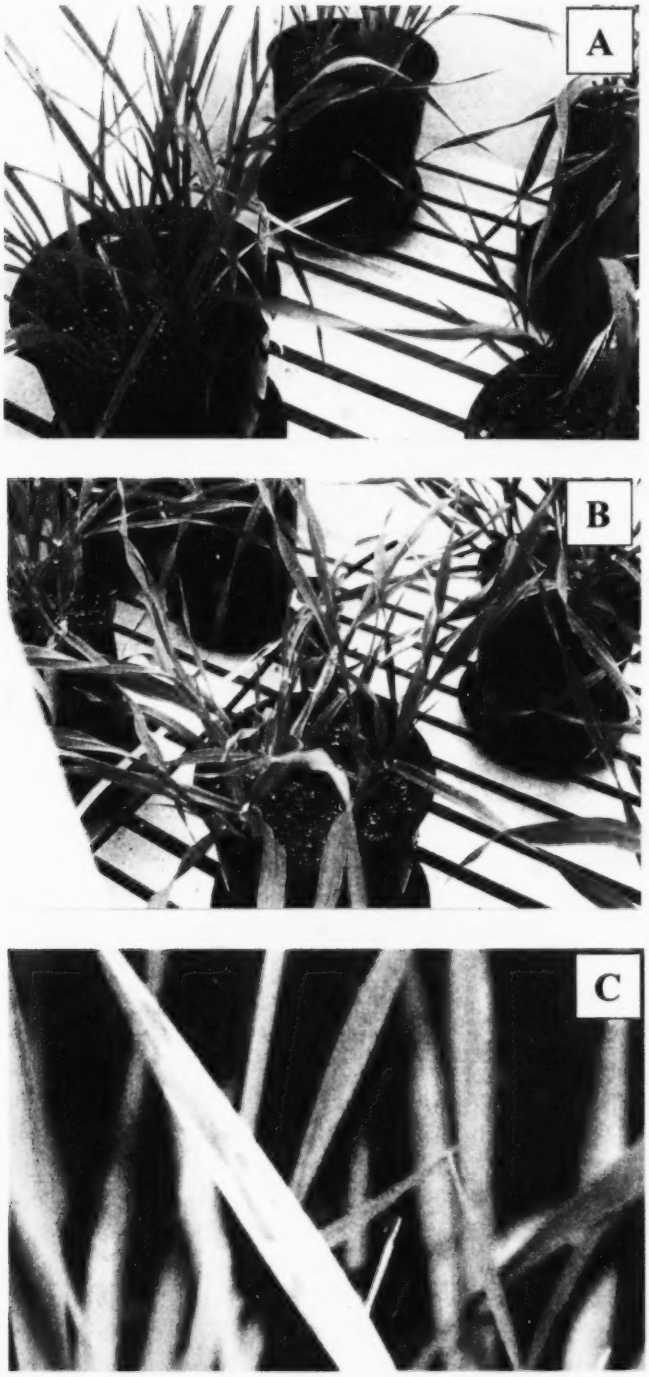

Figure 2 **Symptoms of O_3 injury in wheat cv. CDC Teal (A) and barley cv. AC Lacombe (B) exposed to approximately 50 ppb O_3 from time of emergence. Close-up of leaf of wheat cv. CDC Teal injured by O_3 showing mottled chlorosis (C)**

Table 2 **Percentage of plants per treatment exhibiting visual symptoms of O_3 injury, total leaf area of six largest plants, total leaf biomass of six largest plants for radish cv. Early Scarlet Globe grown in charcoal filtered air (0 ppb) or at approximately 50 ppb O_3 and treated with EDU at either 0, 75 or 150 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	% of plants with O_3 injury	Leaf area (cm^2)	Leaf Biomass (g)
0	0	0.0 ± 0.0	429.1 ± 17.8	2.49 ± 0.04
	75	0.0 ± 0.0	424.7 ± 14.3	2.36 ± 0.17
	150	0.0 ± 0.0	441.2 ± 34.4	2.51 ± 0.57
50	0	5.3 ± 2.7	375.1 ± 16.1	2.29 ± 0.12
	75	0.0 ± 0.0	330.0 ± 2.4	1.93 ± 0.15
	150	0.0 ± 0.0	368.2 ± 20.7	2.15 ± 0.10

Values are means ± SE. N = 3.

Table 3 **Percentage of plants per pot exhibiting visual symptoms of O_3 injury, total leaf area, total leaf biomass and total hypocotyls biomass per pot for radish cv. Cherry Belle grown in charcoal filtered air (0 ppb) or at approximately 50 ppb O_3 and treated with one of three levels of EDU**

O_3 level (ppb)	EDU	% of plants with O_3 injury	Leaf area (cm^2)	Leaf biomass (g)	Hypocotyl biomass (g)
0	0X	0.0 ± 0.0	747.7 ± 32.4	2.65 ± 0.05	2.61 ± 0.38
	1X	0.0 ± 0.0	802.5 ± 13.1	3.07 ± 0.23	3.31 ± 0.60
	3X	0.0 ± 0.0	791.0 ± 40.2	2.76 ± 0.12	2.55 ± 0.58
50	0X	100.0 ± 0.0	805.1 ± 55.9	2.69 ± 0.11	2.33 ± 0.45
	1X	66.7 ± .0.0	772.9 ± 62.3	2.58 ± 0.37	2.56 ± 0.37
	3X	0.0 ± 0.0	783.7 ± 61.6	2.65 ± 0.26	2.42 ± 0.56

0X EDU = controls; 1X EDU = 15 mg/pot, 3X EDU = 45 mg/pot; all treatments applied as a single soil drench. Values are means ± SE. N = 3.

Ozone substantially decreased leaf area, leaf biomass and hypocotyl weights in cv. Early Scarlet Globe but not in Cherry Belle. EDU applications did not affect leaf area, leaf biomass and hypocotyl weight. Hassan *et al.* (1995) showed that EDU application to radishes in the absence of O_3 had no significant effect on growth. Kostka-Rick and Manning (1993) describe dosages of EDU that are effective in protecting radish against O_3 damage without interfering with plant growth itself. The dosages used in the present study were derived from the studies of Kostka-Rick and Manning.

Table 4 **Percentage of plants per pot exhibiting visual symptoms of O_3 injury, total leaf area, total leaf biomass and total hypocotyls biomass per pot for radish cv. Early Scarlet Globe grown in charcoal filtered air (0 ppb) or at approximately 50 ppb O_3 and treated with one of three levels of EDU**

O_3 level (ppb)	EDU	% of plants with O_3 injury	Leaf area (cm^2)	Leaf biomass (g)	Hypocotyl biomass (g)
0	0X	0.0 ± 0.0	795.9 ± 33.0	2.74 ± 0.04	2.26 ± 0.26
	1X	0.0 ± 0.0	772.6 ± 17.8	2.69 ± 0.06	2.72 ± 0.19
	3X	0.0 ± 0.0	679.8 ± 43.9	2.71 ± 0.25	2.41± 0.45
50	0X	100.0 ± 0.0	672.5 ± 57.4	2.29 ± 0.22	1.42 ± 0.47
	1X	33.3 ± .0.0	750.9 ± 50.4	2.55 ± 0.14	1.53 ± 0.33
	3X	0.0 ± 0.0	623.5 ± 35.9	2.50 ± 0.08	1.45 ± 0.30

0X EDU = controls; 1X EDU = 15 mg/pot, 3X EDU = 45 mg/pot; all treatments applied as a single soil drench. Values are means ± SE. N = 3.

3.3.2 Crop Trials

Analysis of variance was used to analyze the data for main effects of ozone, EDU and ozone x EDU interactions. Unless otherwise indicated, statistical significance was at $P \geq 0.95$. Analyses showed main effects of ozone on biomass, head weight, seed number and seed weight in wheat cv. CDC Teal and on the same parameters plus head number and weight per thousand seed in wheat cv. AC Taber (Table 5). Main effects of ozone were only observed for weight per thousand seeds in barley cv. AC Lacombe, biomass, seed number ($P \geq 0.90$) and seed weight for canola cv. Quantum and seed number and weight for canola cv. Sprint (Table 5) while no main effects of ozone were observed for field pea. Main effects of EDU were observed for head weight, seed number and seed weight for in wheat cv. CDC Teal, head weight in wheat cv. AC Taber and barley cv. AC Lacombe ($P \geq 0.90$) and biomass in canola cv. Quantum while no main effects of EDU were observed in canola cv. Sprint and in field pea (Table 5). Main effects of the ozone x EDU interaction were only observed for head weight ($P \geq 0.90$) and seed number in wheat cv. CDC Teal, for head weight ($P \geq 0.90$) in barley cv. AC Lacombe and for weight per thousand seeds for canola cv. Sprint (Table 5). The analyses indicate that wheat cv. AC Taber was the most ozone-sensitive cereal studied, that the sensitivity to ozone of the two cultivars of canola was similar and that field pea was unaffected by ozone levels of about 35 ppb.

Table 5 **Main effects table for wheat, barley and canola. No main effects were detected for field pea**

	Main Effects		
	EDU	Ozone	Ozone x EDU
Wheat cv. CDC Teal			
Biomass		**	
Head weight	**	**	*
Seed number	**	**	**
Seed weight	**	**	
Wheat cv. AC Taber			
Biomass		**	
Head number		**	
Head weight	**	**	
Seed number		**	
Seed weight		**	
Weight / 1000 seeds		**	
Barley cv. AC Lacombe			
Head weight	*		*
Weight / 1000 seeds		**	
Canola – cv. Quantum			
Biomass	**	**	
Seed number		*	
Seed weight		**	
Canola – cv. Sprint			
Seed number		**	
Seed weight		**	
Weight / 1000 seeds			**

*** = significant at $P \geq 0.90$**
**** = significant at $P \geq 0.95$**

In wheat cv. CDC Teal, no effects of O_3 nor EDU on plant height were observed (Table 6). Above-ground biomass was decreased by exposure to O_3 (Figure 3). Effects of EDU on seed weight and seed number were significant at $P \geq 0.90$ and $P \geq 0.95$, respectively. While no effects of O_3 were observed on number of seed heads, O_3 did cause a decrease in head weights, seed number (Table 6) and seed yield (Figure 3) in all treatments. When plants were treated with ozone and EDU, 75 ppm EDU significantly increased ($P \geq 0.90$) head weights, seed number and seed weights, while 150 ppm EDU reduced all three parameters below the 0 EDU treatment. Weight per thousand seeds was unaffected by O_3 and EDU treatment.

In wheat cv. AC Taber, no effects of O_3 nor EDU on plant height were observed (Table 7). Above-ground biomass was decreased by exposure to O_3 (Figure 4). Number of seed heads increased in plants treated with elevated O_3 but weight of heads, number of seeds (Table 7), seed yield (Figure 4) and weight per thousand seeds (Table 7) all decreased suggesting that heads contained sterile or aborted florets and that seeds did not develop fully. Increased number of seed heads may be due to increased tillering which is a stress response observed in cereals exposed to ethylene (Archambault and Li 1999) and O_3 (Mulholland *et al.* 1997). The cultivar

specificity of O_3 effects observed here is not unique to this study, similar findings were described by Slaughter *et al.* (1989). Ojanpera *et al.* (1998) also found that O_3 could cause decreases in seed yield and weight per thousand seeds in wheat. While few EDU effects were observed, alleviation of O_3 effects on head and seed weights were significant at $P \geq 0.90$, but full recovery was never observed. In these experiments results suggest that CDC Teal was more sensitive to EDU than AC Taber, while AC Taber was more sensitive to ozone.

In barley cv. AC Lacombe, no effects of O_3 nor EDU on plant height (Table 8) and above-ground biomass (Figure 5) were observed. The number of seed heads was generally greater in plants grown at elevated O_3 and treatment with 75 ppm EDU increased the number of seed heads in plants grown at low O_3 (Table 8). Weights of seed heads, number of seeds (Table 8) and seed yields (Figure 5) were greater in plants grown at elevated O_3 but addition of EDU did not further enhance growth. In contrast, treatment with EDU at 75 ppm and 150 ppm caused significant increases in weights of seed heads ($P \geq 0.95$) and increases in number of seeds and seed yields at low O_3 levels. Weight per thousand seeds was unaffected by increased O_3 or EDU treatment (Table 8). Experiments need to be repeated to verify the finding that O_3 can, in some instances, increase growth and yield.

Based on the magnitude of O_3 effects and the low efficacy of EDU treatments in the first experiments, O_3 levels were lowered to 35 ppb for the subsequent field pea and canola trials. Ozone had little effect on plant height and above-ground biomass in field pea (Figure 6 and Table 9). Seed yield was decreased by exposure to O_3 and EDU appeared to cause some recovery in yield (Figure 6). None of the other parameters measured were affected by increased O_3 and addition of EDU had little or no effect (Table 9). EDU application had no statistically significant effects on any of the parameters measured.

The two cultivars of canola responded differently to exposure to O_3. While plant height and above-ground biomass were not affected by O_3, seed number and yield increased in cv. Quantum and decreased in cv. Sprint (Figures 7 and 8, Tables 10 and 11). Weight per thousand seeds was unaffected by O_3 (Tables 10 and 11). Effects of EDU were only observed in cv. Quantum where biomass increased significantly ($P \geq 0.95$) when EDU was applied at 75 ppm (Figure 7) and in cv. Sprint where weight per thousand seed increased significantly in plants treated with EDU at 300 ppm (Table 11). In cv. Sprint, some recovery in seed yield occurred (Figure 8) but recovery was incomplete and the effects were not statistically significant.

Table 6 **Vegetative and reproductive parameters of wheat cv. CDC Teal grown in charcoal filtered air (0 ppb) or at approximately 50 ppb O_3 and treated with EDU at either 0, 75 or 150 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	Plant Height (cm)	Number of Heads	Weight of Heads (g)	Number of Seeds	Weight Per 1000 Seeds (g)
0	0	68.7 ± 0.9	38 ± 2	38.7 ± 1.3	898 ± 19	31.8 ± 0.7
	75	67.8 ± 1.0	37 ± 1	41.2 ± 0.3	972 ± 20	31.2 ± 0.4
	150	68.3 ± 0.6	37 ± 1	40.3 ± 0.3	962 ± 3	30.7 ± 0.4
50	0	68.6 ± 0.8	36 ± 1	33.8 ± 0.5	808 ± 8	30.8 ± 0.6
	75	67.5 ± 1.0	38 ± 2	35.3 ± 0.7	848 ± 19	30.8 ± 0.2
	150	69.1 ± 1.0	37 ± 1	30.8 ± 1.8	748 ± 37	30.2 ± 0.7

Values are means ± SE. N = 4.

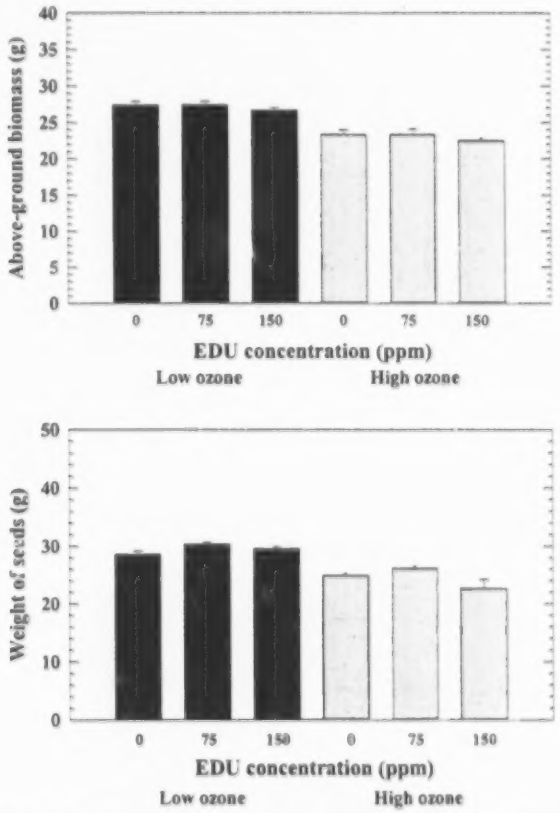

Figure 3 **Above-ground biomass and seed yields of wheat cv. CDC Teal grown in charcoal filtered air (0 ppb – low O_3) or at approximately 50 ppb (high O_3) O_3 and treated with different levels of EDU. Bars are means ±SE. N = 4**

Table 7 **Vegetative and reproductive parameters of wheat cv. AC Taber grown in charcoal filtered air (0 ppb) or at approximately 50 ppb O_3 and treated with EDU at either 0, 75 or 150 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	Plant Height (cm)	Number of Heads	Weight of Heads (g)	Number of Seeds	Weight Per 1000 Seeds (g)
0	0	61.7 ± 5.6	24 ± 1	42.6 ± 0.3	851 ± 22	36.2 ± 0.9
	75	63.4 ± 5.4	26 ± 1	44.6 ± 1.3	862 ± 43	35.9 ± 0.3
	150	63.2 ± 6.0	22 ± 1	42.4 ± 0.2	815 ± 19	37.3 ± 0.5
50	0	61.1 ± 5.5	31 ± 2	29.5 ± 0.3	664 ± 24	29.5 ± 1.5
	75	61.1 ± 5.1	29 ± 1	33.2 ± 1.4	735 ± 29	31.8 ± 0.8
	150	60.3 ± 6.1	29 ± 1	31.7 ± 1.1	705 ± 18	31.2 ± 2.2

Values are means ± SE. N = 4.

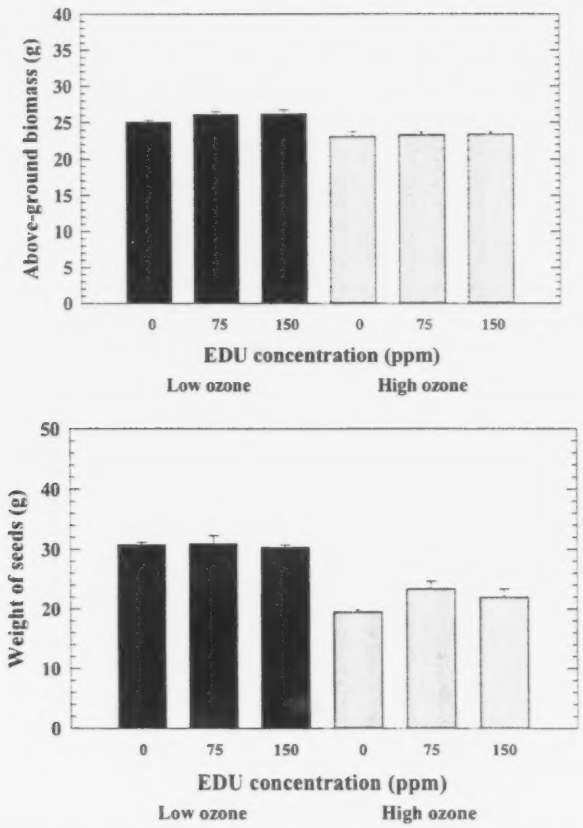

Figure 4 **Above-ground biomass and seed yields of wheat cv. AC Taber grown in charcoal filtered air (0 ppb – low O_3) or at approximately 50 ppb (high O_3) O_3 and treated with different levels of EDU. Bars are means ±SE. N = 4**

Table 8 **Vegetative and reproductive parameters of barley cv. AC Lacombe grown in charcoal filtered air (0 ppb) or at approximately 50 ppb O_3 and treated with EDU at either 0, 75 or 150 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	Plant Height (cm)	Number of Heads	Weight of Heads (g)	Number of Seeds	Weight Per 1000 Seeds (g)
0	0	63.4 ± 1.3	45 ± 3	24.5 ± 0.2	310 ± 13	39.8 ± 0.9
	75	63.6 ± 1.0	54 ± 2	30.7 ± 0.7	428 ± 17	39.0 ± 0.3
	150	64.2 ± 1.1	49 ± 5	30.6 ± 2.7	419 ± 67	40.1 ± 0.7
50	0	64.9 ± 1.2	54 ± 1	29.6 ± 1.3	414 ± 42	38.3 ± 0.8
	75	65.4 ± 1.2	53 ± 2	29.6 ± 1.1	386 ± 24	37.7 ± 0.2
	150	66.0 ± 1.1	51 ± 2	29.2 ± 1.4	415 ± 45	38.2 ± 0.7

Values are means ± SE. N = 4.

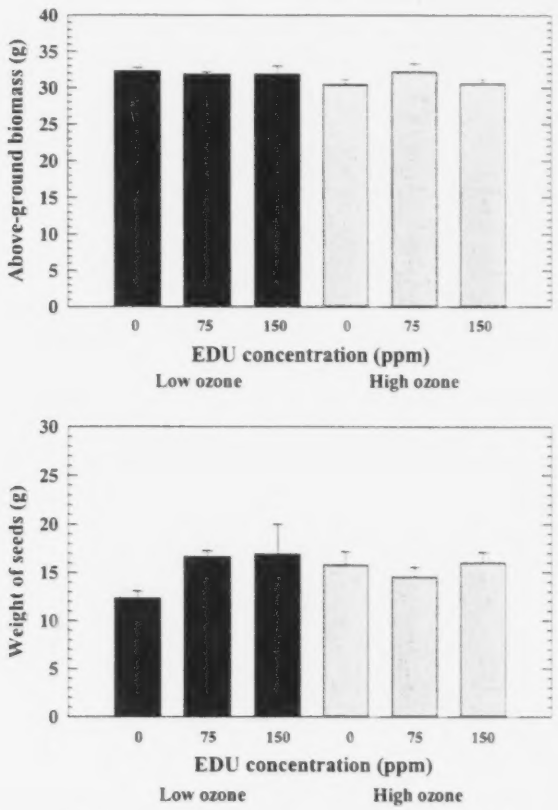

Figure 5 **Above-ground biomass and seed yields of barley cv. AC Lacombe grown in charcoal filtered air (0 ppb – low O_3) or at approximately 50 ppb (high O_3) O_3 and treated with different levels of EDU. Bars are means ±SE. N = 4**

Table 9 **Vegetative and reproductive parameters of field pea cv. Carrera grown in charcoal filtered air (0 ppb) or at approximately 35 ppb O_3 and treated with EDU at either 0, 150 or 300 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	Plant Height (cm)	Number of Pods	Weight of Pods (g)	Number of Seeds	Weight Per 1000 Seeds (g)
0	0	500.3 ± 44.3	7 ± 1	7.86 ± 1.66	25 ± 6	258.4 ± 4.5
	150	545.3 ± 18.4	7 ± 0	7.94 ± 0.85	27 ± 3	242.7 ± 3.4
	300	526.3 ± 32.0	7 ± 0	8.69 ± 0.18	28 ± 1	250.3 ± 5.5
35	0	508.8 ± 19.8	6 ± 0	7.70 ± 0.63	24 ± 1	255.0 ± 7.1
	150	519.5 ± 24.2	7 ± 0	7.90 ± 0.89	26 ± 3	249.8 ± 4.5
	300	518.5 ± 27.5	7 ± 1	8.48 ± 0.92	30 ± 1	223.8 ± 23.7

Values are means ± SE. N = 4

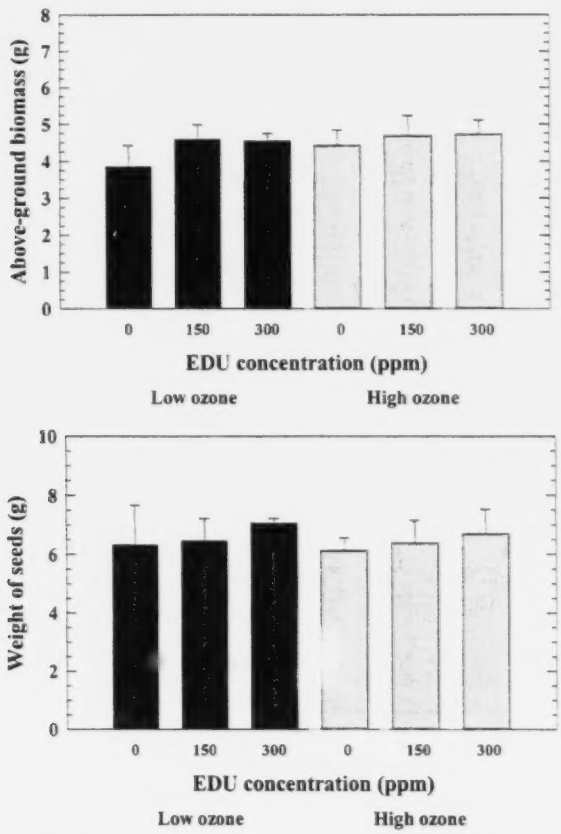

Figure 6 **Above-ground biomass and seed yields of field pea cv. Carrera grown in charcoal filtered air (0 ppb – low O_3) or at approximately 35 ppb (high O_3) O_3 and treated with different levels of EDU. Bars are means ±SE. N = 4**

Table 10 **Vegetative and reproductive parameters of canola cv. Quantum grown in charcoal filtered air (0 ppb) or at approximately 35 ppb O_3 and treated with EDU at either 0, 75 or 150 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	Plant Height (cm)	Number Seeds	Weight Per 1000 Seeds (g)
0	0	126.0 ± 5.1	4368 ± 346	3.26 ± 0.05
	75	128.8 ± 1.2	4766 ± 145	3.42 ± 0.09
	150	124.3 ± 5.7	4573 ± 331	3.31 ± 0.11
35	0	123.0 ± 2.7	5422 ± 305	3.36 ± 0.08
	75	142.3 ± 9.2	4950 ± 237	3.47 ± 0.09
	150	135.0 ± 5.1	4890 ± 430	3.23 ± 0.04

Values are means ± SE. N = 4.

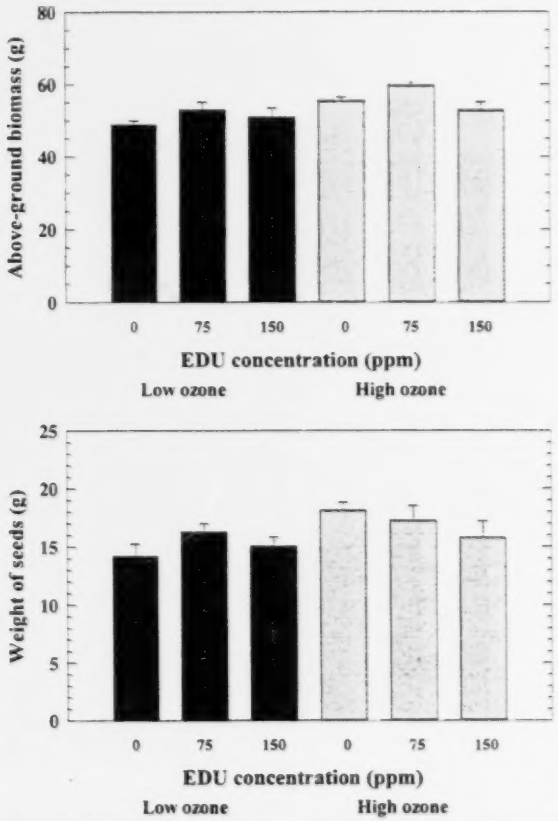

Figure 7 **Above-ground biomass and seed yields of canola cv. Quantum grown in charcoal filtered air (0 ppb – low O_3) or at approximately 35 ppb (high O_3) O_3 and treated with different levels of EDU. Bars are means ±SE. N = 4**

Table 11 **Vegetative and reproductive parameters of canola cv. Sprint grown in charcoal filtered air (0 ppb) or at approximately 35 ppb O_3 and treated with EDU at either 0, 150 or 300 ppm**

O_3 level (ppb)	EDU Conc. (ppm)	Plant Height (cm)	Number of Seeds	Weight Per 1000 Seeds (g)
0	0	135.5 ± 11.4	4544 ± 402	3.67 ± 0.04
	150	133.7 ± 10.6	4553 ± 351	3.45 ± 0.05
	300	138.5 ± 14.9	4548 ± 127	3.83 ± 0.06
35	0	152.5 ± 6.4	3416 ± 161	3.59 ± 0.11
	150	135.0 ± 7.2	3929 ± 431	3.71 ± 0.11
	300	133.3 ± 7.6	4390 ± 318	3.43 ± 0.06

Values are means ± SE. N = 4.

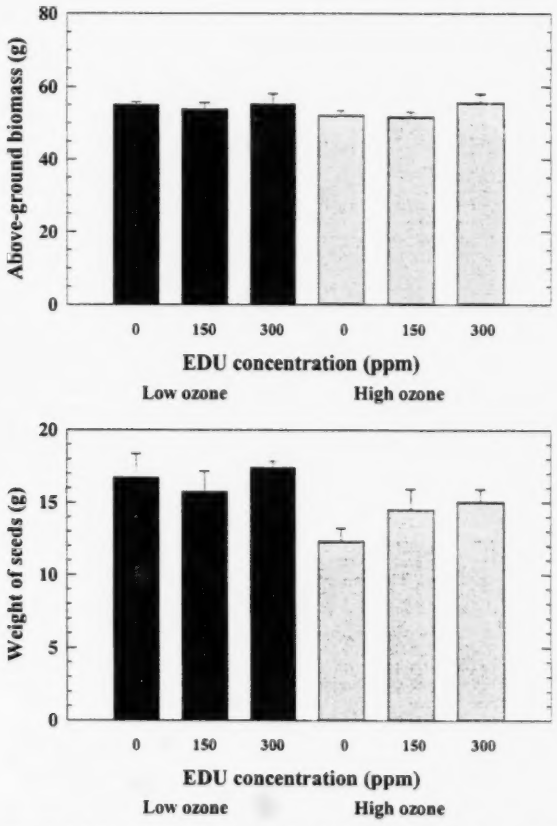

Figure 8 **Above-ground biomass and seed yields of canola cv. Sprint grown in charcoal filtered air (0 ppb – low O_3) or at approximately 35 ppb (high O_3) O_3 and treated with different levels of EDU. Bars are means ±SE. N = 4**

4.0 CONCLUSIONS

The exposure system modified for use in this study performed well. Preliminary experiments using radish produced results that were consistent with those from the literature, confirming that the exposure system and protocols designed for this study were adequate. Of the field crops studied, wheat cv. CDC Teal was found to be most sensitive to EDU while AC Taber was most sensitive to ozone. No evidence of plant growth promoting effects of EDU was found in the absence of O_3. Experiments using barley cv. AC Lacombe need to be repeated to verify that the observed increases in growth and yield were O_3 related and not due to other effects.

5.0 REFERENCES

Archambault DJ and X Li (1999) REPORT II: The effects of ethephon on barley, wheat, oats, field peas and canola: *A screening test for the determination of ethylene sensitivity.* Alberta's Ethylene/Crop Research Project. Report to Alberta Environment. Pp. 84.

Archambault DJ, X Li and JJ Slaski (2000) Ozone protection in plants – The potential use of chemical protectants to measure atmospheric oxidant damage in Alberta crops. ISBN 0-7785-1226-6. Report prepared for the Air Research Users Group, Alberta Environment, Edmonton, Alberta Pp. 43.

Archambault DJ and X Li (2001) Evaluation of Ozone Effects on Crops Using the Anti-oxidant Ethylene Diurea (EDU). Final report. Report to Alberta Environment. Pp. 22.

Foster KW, JP Guerard, RJ Oshima, JC Bishop and H Timm (1983) Differential ozone susceptibility of centennial russet and white rose potato as demonstrated by fumigation and antioxidant treatments. American Potato Journal 60: 127-139.

Hassan IA, MR Ashmore and JNB Bell (1995) Effect of ozone on radish and turnip under Egyptian field conditions. Environmental Pollution 89: 107-114.

Kostka-Rick R and WJ Manning (1993) Dose-response studies with ethylenediurea (EDU) and radish. Environmental Pollution 79: 249-260.

Kostka-Rick R, WJ Manning and JP Buonaccorsi (1993) Dynamics of biomass partitioning in field-grown radish varieties, treated with ethylenediurea. Environmental Pollution 80: 133-145.

Legassicke BC and DP Ormrod (1981) Suppression of ozone-injury on tomatoes by ethylene diurea in controlled environments and in the field. HortScience 16(2): 183-184.

Li X and DJ Archambault (1999) REPORT I: Design and Performance of ARC's Ethylene Exposure System. Alberta's Ethylene/Crop Research Project. Report to Alberta Environment. Pp. 50.

Little TM and FJ Hills (1978) Agricultural experimentation. design and analysis. John Wiley and Sons, Inc. New York. Pp. 350.

Manning WJ (1995) Use of protective chemicals to assess the effects of ambient ozone on vegetation. Proceedings of the 88th Annual Meeting of the Air & Waste Management Association Pp. 12.

Manning WJ and SV Krupa (1992) Experimental methodology for studying the effects of ozone on crops and trees. *In*: Surface level ozone exposures and their effects on vegetation. (AS Lefohn ed.) p. 93-156.

Mulholland BJ, J Craigon, CR Black, JJ Colis, J Atherton and G. Landon (1997) Effects of elevated carbon dioxide and ozone on the growth and yield of spring wheat (Triticum aestivum L.) Journal of Experimental Botany 49(306): 113-122.

Ojanpera K, E Patsikka and T Ylaranta (1998) Effects of low ozone exposure of spring wheat on net CO_2 uptake, Rubisco, leaf senescence and grain fill. New Phytologist 138: 451-460.

Slaughter LH, CL Mulchi, EH Lee and K Tuthill (1989) Chronic ozone effects on yield and grain quality of soft red winter wheat. Crop Science 29: 1251-1255